SHORT COMMUNICATION

Nonlinear relationships and phylogenetically independent contrasts

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Abstract

The method of phylogenetically independent contrasts is commonly used for exploring cross-taxon relationships between traits. Here we show that this phylogenetic comparative method (PCM) can fail to detect correlated evolution when the underlying relationship between traits is nonlinear. Simulations indicate that statistical power can be dramatically reduced when independent contrasts analysis is used on nonlinear relationships. We also reanalyse a published data set and demonstrate that ignoring nonlinearity can affect biological inferences. We suggest that researchers consider the shape of the relationship between traits when using independent contrasts analysis. Alternative PCMs may be more appropriate if data cannot be transformed to meet assumptions of linearity.

Introduction

The use of phylogenetic comparative methods (PCMs) has become standard in studies seeking to identify evolutionary correlations across taxa. These methods address the problem of phylogenetic nonindependence: because taxa may be similar simply due to shared ancestry, comparative data often violate statistical assumptions of independence. One use of PCMs is to draw inferences about the covariation of traits across taxa while taking into account this phylogenetic nonindependence (Felsenstein, 1985; Harvey & Pagel, 1991; Garland & Ives, 2000; Martins, 2000).
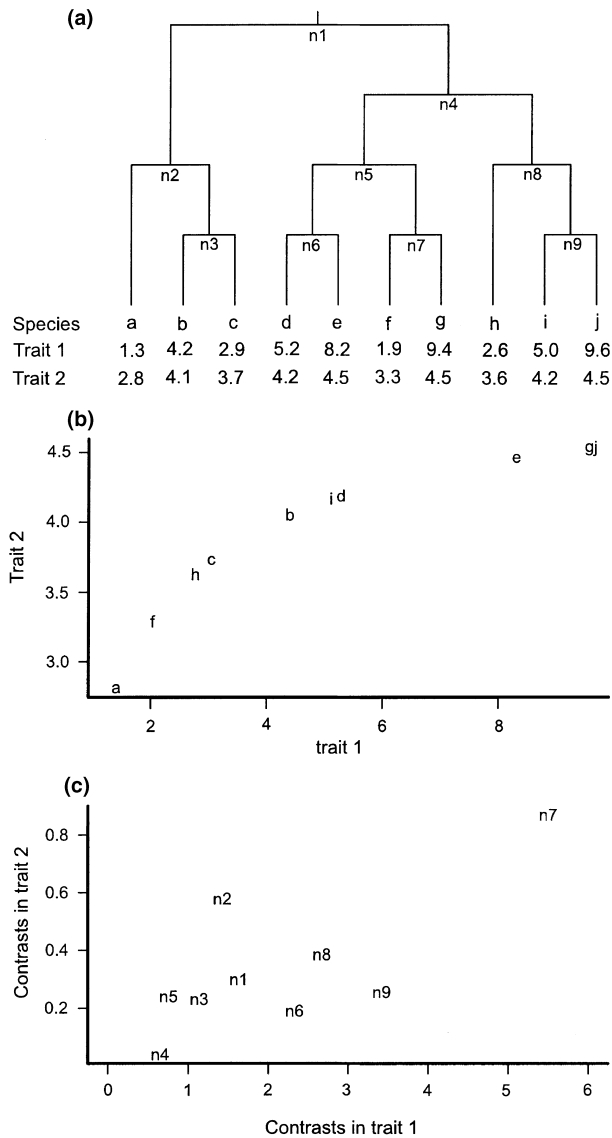
Perhaps the most widely used PCM is phylogenetically independent contrasts (PIC; Felsenstein, 1985; Garland et al., 1992). This method removes the effect of shared evolutionary history by calculating differences in trait values between sister taxa (both extant and ancestral). If

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standardized differences, or contrasts, in one trait significantly covary with contrasts in another trait, then the two traits are evolutionarily correlated. In other words, change in one trait has been accompanied by change in the other. Simulation studies indicate that PIC performs well in a wide variety of situations and under different models of evolutionary change (Martins et al., 2002). However, one important consideration, the shape of the relationship between the traits, has received less attention than it deserves.

In the original formulation of PIC, Felsenstein (1985) assumed that pairs of trait values were drawn from a bivariate normal distribution, leading to a linear relationship between expected values of the traits. If this is the case, contrasts will be linearly related as well, with the same expected slope as the true slope (Harvey & Pagel, 1991). However, if the underlying relationship is nonlinear, difficulties arise in PIC analysis. Harvey & Pagel (1991, Fig. 5.19) point out that a nonlinear relationship between traits may yield a relationship between contrasts that is opposite in sign to that of the true relationship. However, arriving at such a patently false conclusion is prevented by forcing the line relating the contrasts through the origin (Grafen, 1989, 1992; Garland et al., 1992). A more common consequence of nonlinearity in the underlying relationship is an increase in scatter in the relationship between the contrasts.

(a)

Species	a	b	c	d	e	f	g	h	i	j
Trait 1	1.3	4.2	2.9	5.2	8.2	1.9	9.4	2.6	5.0	9.6
Trait 2	2.8	4.1	3.7	4.2	4.5	3.3	4.5	3.6	4.2	4.5

(b)

(c)

Fig. 1 An example of the consequences of a nonlinear underlying relationship. (a) Ten species (a–j) are related as shown in the phylogeny. Each branch segment has a length of either 1 or 2 units. The internal nodes are labelled n1–n9. Values for two variables, traits 1 and 2, are given for each species. (b) Scatterplot of the relationship between the two traits, labelled by species. Note that no particular clade is responsible for the nonlinearity. (c) Plot of standardized contrasts in trait 2 against standardized contrasts in trait 1. Contrasts in trait 1 were positivized for presentation as recommended by Garland *et al.* (1992). The tight underlying relationship in (B) has degenerated into a loose scatter of points, reducing our ability to detect a relationship. The points are labelled by the nodes they represent.

As an example, consider a hypothetical phylogeny of 10 species with values for two traits (Fig. 1a). The underlying relationship between the traits is nonlinear (Fig. 1b), and the relationship between the resulting

contrasts is characterized by much scatter (Fig. 1c). The scatter arises because a given contrast in trait 1 is not associated with a consistent contrast in trait 2. Rather, contrasts in trait 2 are related not only to the magnitude of the contrasts in trait 1, but also to the absolute values of trait 1. An additional source of error is that nodes are assigned values away from the underlying, true line. A consequence of the increase in scatter is a reduction in the statistical power of PIC analyses to detect the true relationship between traits.

Empiricists and theoreticians discussing PIC rarely make explicit the assumption of linearity in the relationship between trait values. Among various descriptions of independent contrasts (Felsenstein, 1985; Burt, 1989; Grafen, 1989; Harvey & Pagel, 1991; Garland *et al.*, 1992, 1999) only Harvey & Pagel explore in any detail the consequences of nonlinearity of the underlying relationship between traits [although Garland *et al.* (1992) discuss nonlinear patterns between contrasts, a different issue]. This lack of emphasis from theoreticians may well be because the problem is obvious to them; unfortunately, empiricists seem to overlook the issue as well. To investigate whether empiricists routinely assess linearity between traits before calculating contrasts, we searched for comparative studies published in 2002 in the journals *Ecology, Evolution, Journal of Evolutionary Biology* and *Proceedings of the Royal Society of London, Series B*. Of 29 papers in which PIC analyses were carried out on continuous traits, only one states that the shape of the relationship between traits was evaluated. It would appear, then, that empiricists do not ordinarily test for linearity.

In this paper, we use computer simulations to demonstrate that when the underlying relationship between two variables is nonlinear, PIC analysis suffers from reduced statistical power. We also reanalyse a published data set to illustrate that this problem can affect inferences drawn from PIC analyses. Finally, we discuss methods of incorporating nonlinearity into phylogenetic comparative analyses.

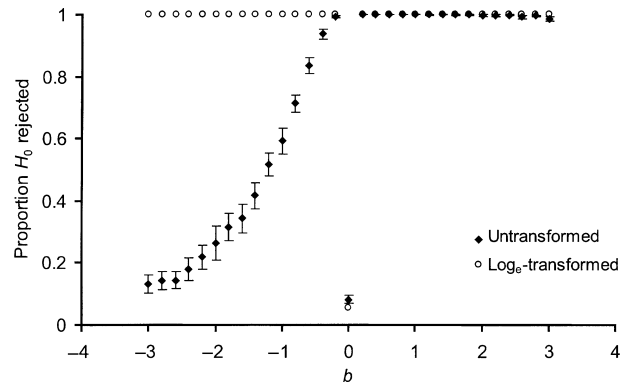## Methods and results

Computer simulations

We used simulation methods to assess the statistical power of PIC when traits are related in a nonlinear fashion. We simulated the evolution of two traits, *x* and *y*, along 15 randomly generated phylogenies, each with 20 extant taxa. The phylogenies were generated in COMPARE 4.4 using branching process models to generate topologies and branch lengths (Martins, 2001). The traits evolve such that they are nonlinearly related, $y = x^b$. Our simulated evolutionary process begins with a single ancestor at the root of the phylogeny and results in phenotypic values for extant taxa at the tips. In order to follow Felsenstein (1985) in modelling the correlated evolution of traits as a Brow-
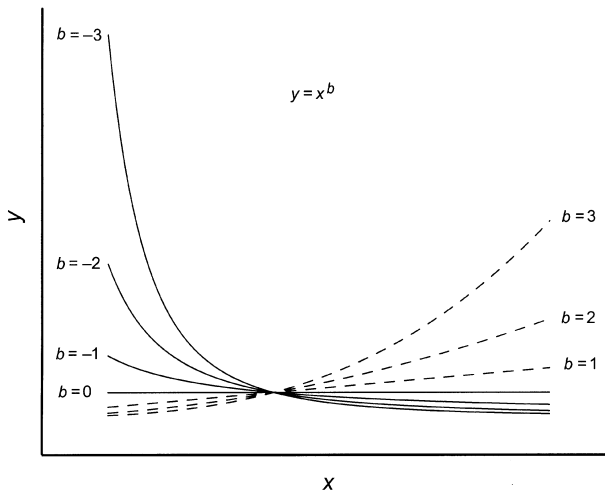
Fig. 2 Shapes of relationships evaluated in computer simulations of the effect of nonlinearity on independent contrasts analyses. Solid lines indicate negative values, and dashed lines indicate positive values of b, which was varied in the simulations from −3 to 3, at intervals of 0.2.

Fig. 3 Statistical power (proportion of runs in which the null hypothesis, H_0, was rejected when $b \neq 0$) of independent contrasts at varying degrees of nonlinearity, both on untransformed (solid diamonds) and $\log_e$-transformed (linearized) data (open circles). Nonlinearity is defined by the parameter b in the relationship $y = x^b$. When $b = 0$, the proportion of runs in which the null hypothesis was rejected is an estimate of type I error. Each point represents a mean across 15 phylogenies; error bars are 95% CIs.

nian motion (BM) process, we assumed that x and y evolve on a log scale. Thus X ($\log_e x$) and Y ($\log_e y$) follow a BM process and are linearly related. At each new generation, X and Y values were drawn from a bivariate normal distribution defined by a vector of mean values (the trait values in the previous generation) and a variance–covariance matrix, which determines the slope of Y on X. By varying this slope, we are in effect varying b (and thus the degree of nonlinearity) in the original relationship $y = x^b$. When $b = 1$, the relationship between y and x is linear, and when $b = 0$, there is no relationship between the variables (i.e. the null hypothesis is true). We varied b between −3 and 3 in increments of 0.2 by changing the covariance between X and Y and the variance in Y, according to the standard relationship between slope, variances and covariance. The per-generation variance in X was kept constant at 0.01, as was the correlation between X and Y, at 0.8. Figure 2 illustrates the shapes of the different nonlinear relationships we used.

For every value of b we performed 100 simulation runs along each of the 15 random phylogenies. All trees were standardized to a total branch length of 100 generations from root to tip. In each run, we assigned starting (root) values of (0, 0) to X and Y. At the end of each run we created raw trait values by exponentiating X and Y for the tip taxa. We performed PIC analyses on both the raw (x, y) and the log-scale (X, Y) trait values using the 'ape' package of Paradis *et al.* (2002) in the statistical and programming language R, version 1.6 (Ihaka & Gentleman, 1996). Contrasts obtained in this way were identical to those calculated using COMPARE 4.4. For each run, we checked that contrasts in both raw and log-

transformed traits were adequately standardized by regressing the absolute values of the contrasts on their expected standard deviation (the square root of the sum of the branch lengths: Felsenstein, 1985; Garland *et al.*, 1992). We then assessed the relationship between contrasts using least-squares linear regression through the origin (Garland *et al.*, 1992; Rohlf, 2001) at a significance level of 0.05.

The results of our exploration of the effect of nonlinearity on the performance of PIC are illustrated in Fig. 3. There was a drastic reduction in the statistical power of PIC as b became more negative; even a mildly nonlinear negative function ($b = -1$) yielded a statistical power of only about 0.6, which means that in approximately 40% of runs PIC analysis did not detect a true relationship at this value of b. Positive values of b did not have as drastic an effect, although statistical power did decline slightly at extreme values (Fig. 3). Log-transformation of the traits solved the problem; statistical power after transformation was 1 regardless of the sign or magnitude of b. Thus, as expected, PIC always detected a true relationship after log-transformation of variables related through a power function. Type I error (the proportion of runs in which the null hypothesis was rejected when $b = 0$), averaged across the 15 phylogenies, was close to that expected for both untransformed traits [mean ± 95% confidence interval (CI): 0.08 ± 0.01] and log-transformed trait values (0.05 ± 0.01).

An empirical example

We illustrate the effect of nonlinear relationships on biological inferences with a reanalysis of a published data set on morphological and ecological variables across 35

genera of birds of prey (raptors) in the family Accipitridae (Krüger, 2000). Krüger found that several significant correlations between these variables disappeared after PIC analysis. Using his data (see Appendix in Krüger, 2000 and phylogeny in Holdaway, 1994) we reanalysed the relationship between raptor population density and prey mass. One would expect population density of predators to decline with prey mass, and because energy requirements scale allometrically with body mass, the relationship may well be nonlinear. This appears to be the case (Fig. 4a). A linear regression describes the data poorly, as seen from a plot of the residuals from a linear regression against prey mass (Fig. 4b). If a linear regression were appropriate, residuals should be scattered at random around zero and should show no pattern when plotted against the independent variable. Instead, large deviations from zero are apparent at low values of prey mass, and these deviations are asymmetrical (many small negative residuals and a few large positive residuals). Log_e-transformation of the data appears to linearize the

relationship satisfactorily (Fig. 4d) as shown by the corresponding residual plot (Fig. 4e).

We carried out PIC analyses on the raw variables, as well as on the log_e-transformed variables. Contrasts were satisfactorily standardized in both cases (according to the criterion of Garland *et al.*, 1992) and, as in the simulations, we evaluated the relationships between standardized contrasts by performing least-squares linear regression through the origin.

Contrasts derived from untransformed variables were not significantly related (Fig. 4c; $y = -0.0028x$, $R^2 = 0.07$, $F_{1,33} = 2.66$, n.s.). This would lead one to the conclusion that changes in the population density of raptors are not evolutionarily correlated with changes in the mass of their prey. However, contrast analysis on the log-transformed variables revealed a clear association between population density and prey mass ($y = -0.326x$, $R^2 = 0.26$, $F_{1,33} = 11.64$, $P < 0.05$; Fig. 4f). Thus, when the data are appropriately analysed, we find the expected negative relationship between the two variables.
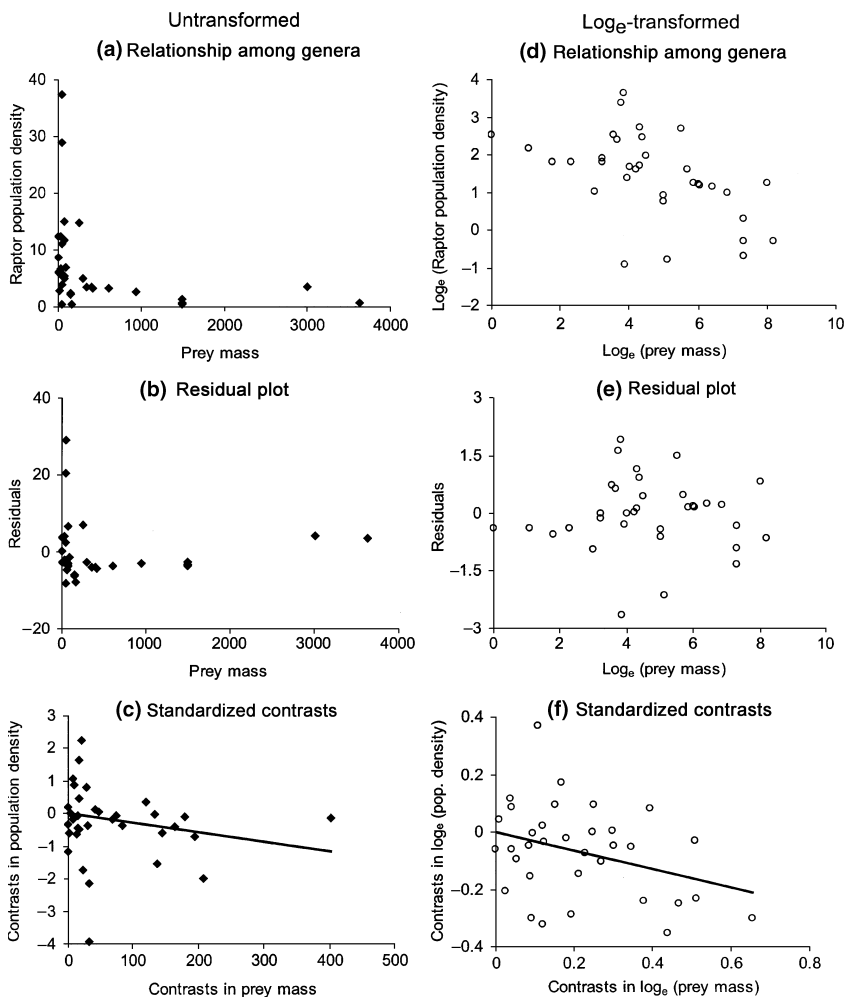
Fig. 4 Reanalysis of the relationship between raptor population density and prey mass from Krüger (2000). Plots of the across-genus relationship (a, d), residuals (b, e), and the relationship between contrasts (c, f: *x*-axis positivized) based on untransformed (left) and log_e-transformed (right) genus values.

Discussion

Our simulation results indicate that the shape of the relationship between traits can affect PIC analyses. Using a simple power function we have shown that when variables are related in a nonlinear manner, the relationship between the contrasts suffers from high scatter and the power to detect a significant association declines. The reduction in statistical power is dramatic for negative relationships because these are particularly nonlinear in our simulations (Fig. 2). However, when relationships are linearized PIC works well, as demonstrated both by the simulations and a reanalysis of a published data set. Based on these findings, we conclude that researchers who use PIC to investigate the correlation between traits should evaluate the shape of the relationship, and that this is an important step in the analysis.

Detecting nonlinear relationships between traits

In many cases, we may predict beforehand that two traits will be nonlinearly related. The pattern that comparative biologists can most commonly expect is perhaps allometry described by power functions of the form $y = a + bx^c$. For example, one would clearly expect such a scaling relationship between a linear measure of body size (such as total length) and a cubic measure (such as mass). These functions are well established as describing relationships among morphological and physiological traits as well as ecological and life-history variables (e.g. Harvey & Pagel, 1991; Charnov, 1993; Enquist *et al.*, 1999; Hendriks, 1999; Garland & Ives, 2000). However, there may often be no explicit theoretical expectation of an allometric relationship between two traits, in which case there is no *a priori* reason for a power function and the pattern must be evaluated empirically (Harvey & Pagel, 1991).

There are other cases in which we might expect nonlinear relationships to arise. For example, when predicting that a trait reaches a maximum at a particular body size (e.g. reproductive power: Jones & Purvis, 1997), one may expect a quadratic relationship. In other cases, physiological and evolutionary constraints may impose limits that preclude linear relationships between traits, and nonlinear relationships should be expected when traits have lower or upper bounds (e.g. gestation time can never be negative; proportions are bounded by zero and one). In such cases, the relationship may be better described by loglinear or logistic functions, and appropriate transformations can be used to produce a linear relationship (see below).

When there is no prior expectation for the shape of the function relating the traits, detecting nonlinearity is complicated by nonindependence of species values. For example, it is important to check whether any apparent nonlinearity is caused solely by a single clade in the database. If this is not the case, standard diagnostics can be used to evaluate the shape of the line. Residuals from a linear regression may be plotted against the *x*-variable; systematic changes in the sign and scatter of residuals indicate that *y* may be nonlinearly related to *x* (Fig. 4; Sokal & Rohlf, 1995; Zar, 1999). In addition to residual analysis, the fit of alternative linear and curvilinear models to the data can be compared statistically (Sokal & Rohlf, 1995, section 16.6). The data are still nonindependent (because these are species values), so the diagnostics described above should be used with caution.

An underlying nonlinearity may sometimes be detectable in the form of an apparent nonzero intercept in the relationship between the contrasts (Harvey & Pagel, 1991; but see Grafen, 1992). However, nonlinearity most often leads to an increase in scatter (e.g. Fig. 1) in the contrasts, and not to a nonzero intercept, so this is not an adequate diagnostic. In addition, because the primary consequence is to increase scatter in the relationship between contrasts, searching for a curvilinear relationship between contrasts (Garland *et al.*, 1992) is not a sufficient test. A more promising method was used by Jones & Purvis (1997) to detect changes in the slope of the line relating two traits. In this method the ratio of the *y*-contrast to the *x*-contrast is calculated for each node (Jones & Purvis call this the contrast slope). Then the contrast slope is plotted against the mean *x*-value of the taxa being compared. If the underlying relationship is linear there should be no change in the contrast slope with the *x* variable. However, if such a plot shows an increasing or decreasing pattern this is evidence for nonlinearity in the underlying relationship. Garland *et al.* (1992) describe a similar method in which the regression between the contrasts includes one or both *x*-values (or their sum) as covariates.

Finally, when data are not continuous (e.g. ranks), the implementation of standard PIC is problematic. With such data, there is no method to test whether the true relationship between traits is linear because the distances between successive ranks may be arbitrary and inconsistent. As a consequence, the analysis of ordinal data using ordinary PIC analyses is unwise. A modified procedure may sometimes be used for analysing rank and categorical data (Purvis & Rambaut, 1995).

Using PIC when relationships are nonlinear

Many curvilinear relationships can be evaluated with PIC if they can be transformed. Allometric power functions can be linearized by log-transforming both variables (Harvey & Pagel, 1991; Garland *et al.*, 1992). Allometric relationships are also likely to have multiplicative and log-normally distributed error. If so, log–log transformation will also yield appropriate normal errors. Exponential relationships ($y = ae^{bx}$) can be similarly linearized by log-transforming the *y*-variable. When appropriate, other transformations (e.g. square root transformation of counts or arcsine square root transformation of propor-

tions) can be used (Sokal & Rohlf, 1995; Zar, 1999). As before, species values cannot be considered independent of one another and a valuable exercise would be to check whether all clades in the analysis show roughly the same pattern after transformation (Grafen, 1989). Data transformations have also been advocated to ensure that Brownian assumptions are satisfied and that resulting contrasts are phylogenetically independent (Purvis & Rambaut, 1995; Freckleton, 2000). Thus, whatever transformation used one must ensure both a linear underlying relationship and independence of contrasts. Finally, not all nonlinear relationships can be transformed to meet the assumptions of PIC. For such relationships (e.g. quadratic curves), alternative comparative methods may be preferable.

Alternatives to PIC

Other techniques for the comparative analysis of continuous data may sometimes be more appropriate than PIC. Some of these methods allow the relationship between traits to be described by a curvilinear function while incorporating phylogenetic nonindependence into a covariance matrix in the error term. Certain curvilinear relationships with known error structure can be analysed with generalized linear models (GLMs). Martins & Hansen (1997) discuss a method of incorporating phylogeny into GLMs, and they demonstrate this using a generalized least squares (GLS) approach. The GLS method can be thought of as an extension of PIC (Grafen, 1989; Martins & Hansen, 1997; Garland & Ives, 2000; Rohlf, 2001) in which one can model how the expected similarity between species declines as their phylogenetic separation increases. This method offers substantial flexibility in model specification (Grafen, 1989; Martins & Hansen, 1997). For example, the fit of alternative models (e.g. $y = x$ vs. $y = x^2$ or $y = x + x^2$) can be compared to evaluate whether there is significant nonlinearity in the relationship. A similar technique, generalized nonlinear least squares (Pinheiro & Bates, 2000), can be used for more complicated curvilinear relationships. In addition to least-squares methods of incorporating phylogenetic information into comparative studies, the use of generalized estimating equations has recently been proposed (Paradis & Claude, 2002). This approach can be used to model the evolution of traits that are expected to have Poisson or binomial distributions, for example counts bounded by zero, or dichotomous traits (e.g. bristle number or presence/absence of a male ornament, respectively).

New methods for analysing interspecific data are continually being developed and tested (reviewed in Martins & Hansen, 1996, 1997; Martins, 2000; Freckleton *et al.*, 2002; Martins *et al.*, 2002; Blomberg *et al.*, 2003), and are increasingly implemented in software written for comparative biologists (e.g. CAIC, Purvis & Rambaut, 1995; PDAP, Garland *et al.*, 1999; COMPARE, Martins, 2001; ape, Paradis *et al.*, 2002). Although PIC is easy to understand and relatively simple to perform, we encourage comparative biologists to explore alternative methods. These alternatives typically allow some assumptions of PIC (e.g. linearity, BM evolution) to be relaxed. However, the ability of these methods to deal with various kinds of nonlinearity has not been treated in detail (but see Martins & Hansen, 1997).

Conclusions

We have shown that by ignoring the shape of the relationship between variables, biologists using PIC methods run the risk of failing to uncover evolutionary patterns. However, nonlinear relationships are not merely obstacles in analysis – they can also provide insights into evolutionary mechanisms. We believe that the goals of PCMs are not simply to determine the 'significance' of relationships but also to determine their form, as this may help generate hypotheses about constraints and causal mechanisms. We suggest that biologists incorporate theoretical and empirical information about the shape of these relationships into comparative studies.

Acknowledgments

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